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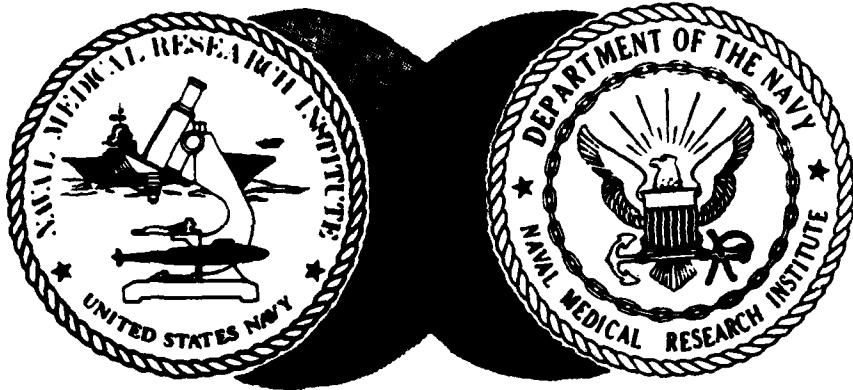
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PRODUCTION AND CELLS WITH SURFACE IGG.

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POLYCLONAL ACTIVATION OF THE MURINE IMMUNE SYSTEM BY AN ANTIBODY TO IgD

II. Generation of Polyclonal Antibody Production and Cells with Surface IgG[1]

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In the accompanying paper we showed that the injection of BALB/c mice with 800 μg of GaMδ induced a direct polyclonal increase in B cell proliferation as well as an indirect increase in T cell proliferation. This led us to investigate whether the same treatment might lead to polyclonal Ig secretion. We found that 6 to 7 days after GaMδ injection one-quarter to one-half of splenic B lymphocytes lost detectable surface IgM and acquired surface IgG. Surface labeling experiments established that much of this IgG had the electrophoretic characteristics of membrane rather than serum IgG, and was, therefore, intrinsic rather than cytophilic. GaMδ also induced a striking increase in Ig secretion 6 to 7 days after injection, as indicated by 1) an approximately eightfold increase in serum IgG1 levels and smaller increases in serum IgM and IgG2a levels; 2) greater than fivefold and 50-fold increases in the in vitro incorporation of ^{3}H-leucine into IgM and IgG, respectively, by spleen cells from GaMδ-injected mice; and 3) three to 10-fold and 20 to 50-fold increases in the percentages of spleen cells with large amounts of intracytoplasmic IgM and IgG, respectively. The great majority of the increase in cell surface and secreted IgG was accounted for by an increase in the IgG1 subclass. Both absorption and plaquing studies indicated that the increase in Ig secretion was polyclonal rather than a specific immune response to goat Ig. The injection of anti-δ antibodies failed to induce B cells from congenitally athymic mice or mice that were tolerant to the injected anti-δ antibody to undergo a switch in cell surface isotype or to differentiate into antibody-secreting cells. We interpret these data and data presented in the companion paper as suggesting the binding to and cross-linking of B cell surface IgD by ligand leads to 1) direct activation of B lymphocytes that can include proliferation; 2) an indirect activation of T lymphocytes that can recognize the ligand as foreign; and 3) stimulation of activated B lymphocytes by T cell and/or accessory cell produced helper factors to undergo a switch in surface Ig isotype and to differentiate into antibody-secreting cells. The injection of mice with heat-aggregated goat IgG or a rat antibody to ThB failed to estimate a switch in surface Ig isotype or the differentiation of B cells into Ig-secreting cells. Thus, the anti-δ-induced events probably involve specific triggering of B cells by a surface Ig-ligand interaction rather than simply the binding of ligand to the B cell. After the catabolism of injected GaMδ there is a rapid loss of surface IgG$^+$ and Ig-secreting cells that appears to be a consequence of cell death and that causes the spleen to return to a condition approximating its prestimulated state.

In vitro studies have demonstrated that anti-immunoglobulin (Ig) antibodies can directly stimulate murine B lymphocyte proliferation (1-9). Moreover, in the presence of supernatants of lectin-stimulated T cells and macrophages, anti-Ig antibodies can induce the differentiation of B lymphocytes into antibody-secreting cells (10-12). In the absence of such stimulated macrophage/T cell supernatants, however, anti-μ and anti-δ antibodies fail to stimulate the in vitro generation of antibody-secreting cells, and, in fact, inhibit the in vitro antigenic stimulation of the differentiation of B cells into cells secreting specific antibodies (12-18). In the accompanying paper, we established that anti-δ antibodies can induce B cell proliferation in vivo under physiologic conditions. In addition, we found that this antibody induced two distinct waves of in vivo lymphocyte proliferation: an initial wave that involved B but not T cells and was neither carrier-dependent nor T-dependent, and a second wave that involved both B and T cells and was both T-dependent and carrier-dependent. The T-dependent nature of the second wave of lymphocyte proliferation suggested that the in vivo injection of anti-δ antibodies might, by indirectly inducing polyclonal T lymphocyte activation, stimulate the polyclonal differentiation of B lymphocytes into antibody-secreting cells. The studies reported here demonstrate that 6 to 7 days after goat anti-mouse-δ (GaMδ)[2]-injection, large increases in the percentage of spleen cells with surface (s) IgG and intracytoplasmic (c) IgG are observed, as well as many fold increases in serum IgG1 levels and in the polyclonal secretion of IgG. These effects are both T-dependent and carrier-dependent.

[2] Abbreviations used in this paper: BCF$_1$, (BALB/c × C57BL/6)F$_1$ mice; c. intracytoplasmic; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; GaF and GaMδ, goat antibodies to ferritin and mouse IgD; GPaRIg, guinea pig anti-rabbit Ig antibody; NI G IgG, normal goat IgG; RaKLH, RaMδ, RaMγ, RaMγ_1, RaMγ_2, RaMγ_3, RaMIg, RaMμ, rabbit antibodies to keyhole limpet hemocyanin and to mouse IgD, IgG (not subclass specific), IgG1, IgG2, IgG3, Ig (not class specific), and IgM; RaThB, rat antibody to the mouse ThB cell surface antigen; s, cell surface; Staph A, protein A bearing Staphylococcus aureus, 4.22aMδ^*, a monoclonal mouse allo-anti-mouse IgD antibody secreted by clone 10-4.22; ELISA, enzyme-linked immunosorbent assay.

MATERIALS AND METHODS

With the following exceptions, the materials and methods used in this study are described in the companion paper.

Antibodies. MOPC-31c (IgG1κ), 10-4.22 (IgG2aκ), FLOPC-21 (IgG3κ), MOPC-195s (IgG2bκ), J606 (IgG3κ), and MOPC-21a (IgG1κ) were purified from hybridoma supernatant or ascitic fluid by fractional elution from protein A-Sepharose columns with pH 6.0, pH 4.5, and pH 3.5 0.1 M citrate buffers (19). Rabbits were immunized twice at monthly intervals with 100 μg of MOPC-31c, 10-4.22, or FLOPC-21 in complete Freund's adjuvant (CFA) to produce anti-IgG1, anti-IgG2, and anti-IgG3 antisera, respectively. Before absorption and affinity purification 50% saturated (NH₄)₂SO₄ cuts of each serum were dialyzed against 0.1 M sodium acetate, pH 4.5, and digested with pepsin (1 mg/50 mg protein) for 18 hr at 37°C. Peptic digests were dialyzed against 0.1 M Tris, pH 8.3, and then were centrifuged for 1 hr at 100,000 × G. The peptic digest of anti-IgG1 antiserum was first absorbed twice with TEPC-183- (IgMκ) Sepharose, MOPC-195s-Sepharose, and J606-Sepharose and then was affinity-purified by absorption to and 3.5 M MgCl₂ elution from MOPC-21a-Sepharose. The eluate, RaMγ₁, was dialyzed against 0.1 M Tris, pH 8.3, and was absorbed with protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) to remove Fc fragments and undigested antibodies. F(ab')₂ fragments of affinity-purified anti-mouse γ₂ antibody (RaMγ₂) were similarly prepared from anti-IgG2 antiserum by absorption with Sepharose-bound TEPC-183, MOPC-21a, and J606, followed by affinity purification with MOPC-195s-Sepharose and protein A-Sepharose absorption; F(ab')₂ fragments of affinity-purified anti-mouse γ₃ antibody (RaMγ₃) were prepared from anti-IgG3 antiserum by absorption with Sepharose-bound TEPC-183, MOPC-21a, and MOPC-195s, followed by affinity purification with J606-Sepharose and protein A-Sepharose absorption. The antibodies prepared were subclass-specific by both Ouchterlony and ELISA² analysis. The F(ab')₂ fragment of affinity-purified guinea pig anti-rabbit Ig (GPaRIg) was prepared as previously described (20) and was absorbed by passage over a column of normal mouse serum bound to CNBr-activated Sepharose (Pharmacia Fine Chemicals). RaMγ₁, RaMγ₂, GPaRIg, and undigested normal goat IgG (NI G IgG) were labeled with fluorescein isothiocyanate (FITC) to molar F:P ratios of from 0.9 to 1.4. Three antisera that bound Ia specificities on B lymphocytes of BALB/c mice were used. A.TH anti-A.TL was prepared as previously described (21). The monoclonal IgG2a produced by hybridoma 25-9.17 (a gift of Drs. David Sachs and Keiko Ozato, National Cancer Institute, National Institutes of Health, which has specificity for determinant 8 on I-A of the b and d haplotypes (22), and the monoclonal IgG2 produced by hybridoma MKD6 (a gift of Drs. John Kappler and Phillipa Marrack, National Jewish Hospital, Denver, CO) that binds to an I-A determinant of the d haplotype (23), were prepared as originally described and were FITC-labeled for direct fluorescence staining.

Immunofluorescence staining for surface antigens. Spleen cell suspensions were stained directly for surface markers with FITC-labeled antibodies as previously described (24). In some experiments cells were stained indirectly with 2.5 μg/ml of the F(ab')₂ fragment of RaMγ₃ (100 μl/2 × 10⁶ cells; 4°C; 30 min) followed after washing with 10 μg/ml of FITC-GPaRIg (same conditions) or with a 1/100 dilution of ultracentrifuged A.TH anti-A.TL antiserum (same conditions) followed by FITC-RaMγ as previously described. RaKLH² was used as a control for staining with RaMγ₃; ultracentrifuged normal mouse serum was the control for A.TH anti-A.TL. Stained cell suspensions were analyzed or sorted with an FACS II fluorescence-activated cell sorter (Becton Dickinson, Mountain View, CA) (24-26).

Fluorescence staining for cIg. Aliquots (0.5 ml) of a spleen cell suspension (5 × 10⁶ cells/ml) in Hanks' balanced salt solution containing 10% newborn calf serum and 0.2% NaN₃ were centrifuged onto glass slides with a Shandon Southern Cytospin cytocentrifuge (Shandon Southern Instruments, Sewickly, PA) for 5 min at 800 rpm. Slides were air-dried, fixed for 30 min in absolute methanol at 4°C, dried again, and then stained for 30 min at room temperature in a humid chamber with FITC-NI G IgG or FITC-labeled affinity-purified F(ab')₂ fragments of RaMμ, RaMγ, RaMγ₁, or RaMγ₂ (all at 100 μg/ml or with the unfluoresceinated F(ab')₂ fragment of affinity-purified RaMγ₃ (25 μl/ml) followed after washing by FITC-GPaRIg (100 μg/ml). Stained slides were washed three times for 5 min each with PBS and one time with deionized water. Slides were then air dried, mounted under glycerol, and examined by fluorescence microscopy using a Leitz Ortholux phase contrast/fluorescence microscope with 12.5× eyepieces and a 63× 1.4 NA oil immersion objective for enumeration of cells with bright intracytoplasmic fluorescence. At least 500 cells on each slide were examined. In preliminary experiments cytocentrifuge preparations were made of IgG1, IgG2a, IgG2b, IgG3, IgM, and IgA-secreting plasmacytoma cells and hybridoma cells (gifts of Dr. Michael Potter and Dr. Philip Fox, NIH) and were stained for cIg. Positive staining was seen only when the appropriate FITC-labeled antibody was used, and IgG2a and IgG2b-secreting cells were stained equivalently by the FITC-RaMγ₃ reagent.

Quantitation of serum Ig levels. Levels of IgM, IgG1, IgG2a, IgG2b, and IgA in mouse sera were analyzed by the radial immunodiffusion technique with Mancini plates purchased from Meloy (Springfield, VA).

Quantitation of IgM, IgG, and total protein secretion. Spleen cells (2 ×

10⁷) were cultured overnight at 37°C in 5 ml of leucine-free medium to which 0.1 mCi/ml of ³H-leucine (Schwarz-Mann, Orangeburg, NY) had been added. Media and culture conditions were as described (27) except that cells were cultured in Costar cluster dishes (Costar, Cambridge, MA) instead of culture tubes. Cells were removed from supernatants by centrifugation, after which supernatants were dialyzed against 0.1 M Tris, pH 8.3. Two milliliter aliquots of dialyzed supernatant were incubated with RaMIg plus protein A-bearing *Staphylococcus aureus* (Staph A) (28). The ³H-labeled Ig-RaMIg-Staph A complexes were then washed and treated for 5 min in Laemmli sample buffer plus 2-mercaptoethanol to elute and reduce the ³H-labeled Ig. The eluted, reduced Ig was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (29). The gels were then fractionated with a Gilson gel mincer (Gilson Medical Electronics, Middleton, WI), and the counts per minute of ³H activity were determined by scintillation spectroscopy. Total protein-associated radioactivity in 25-μl aliquots of cell supernatants was determined by trichloroacetic acid precipitation and scintillation spectroscopy as previously described.

Analysis of sIg. Lactoperoxidase-catalyzed ¹²⁵I surface membrane-labeling of normal spleen cells, NP-40 detergent cell extraction, and immunoprecipitation of antigens with antibodies and Staph A have been described (28), as have the conditions for slab SDS-PAGE, the staining of gels to reveal the positions of standards and unlabeled proteins, and autoradiographic detection of ¹²⁵I-labeled proteins (30).

RESULTS

Effects of anti-δ antibodies on cell surface molecules. As shown in the accompanying paper, injection of GaMδ rapidly removed almost all sIgD from splenic B lymphocytes while having little immediate effect on the percentage of sIgM⁺ spleen cells (Table I). The percentage of sIgD⁺ spleen cells and the fluorescence intensity of positively stained cells remained low until injected GaMδ was no longer detectable in mouse serum (day 10 after injection). Also, as previously noted (31), the fluorescence intensity of sIa⁻ cells stained for this surface marker was greatly increased. These changes were accompanied by an increase in the percentage of sIa⁺ spleen cells and a concomitant decrease in the percentage of Thy-1.2⁺ spleen cells 2 to 3 days after GaMδ injection, and are presumably the result of the early stimulation of B cell but not T cell proliferation by GaMδ. By 5 days after GaMδ injection the percentages of sIa⁺ and sThy-1.2⁺ spleen cells from the GaMδ-treated and control mice were similar, but 6 to 7 days after GaMδ injection spleens from anti-δ-treated mice again showed increased percentages of sIa⁻ and decreased percentages of sThy-1.2⁺ cells (Table I). Similar results were found in three separate experiments. These findings are compatible with either an increase in T cell proliferation or an increase in B cell death at day 5, and a further increase in proliferation of B cells above that of T cells at day 7 after GaMδ injection.

By 6 to 7 days after GaMδ injection an increased percentage of sIa⁻ IgM⁻ cells is seen that is accompanied by a striking increase in the percentage of cells with sIgG (Table I). The appearance of increased numbers of sIgG⁻ spleen cells was dependent on the presence of T lymphocytes and on the recognition of the anti-δ molecule as foreign. Congenitally athymic (nu/nu) mice had only a slight increase in the percentage of sIgG⁺ spleen cells in one experiment (Table I) and had no increase in a second experiment (data not shown). Normal mice made tolerant to goat IgG before injection of GaMδ also failed to have an increase in splenic sIgG⁺ cells 7 days after GaMδ injection (Table I). In addition, whereas BALB/c mice, which have sIgD and sIgG of the a allotype, showed an increase in the percentage of sIgG⁺ spleen cells 7 days after injection of 800 μg of 4.22aMδᵃ (an IgG2a of the b allotype that binds IgD of the a allotype), injection of 4.22aMδᵃ into (BALB/c × C57BL/6)F₁ (BCF₁) mice, which have equal numbers of B cells with sIgD of the a or b allotype and serum IgG of both the a and b allotypes, failed to induce an increase in the percent of sIgG⁺ spleen cells (Table I).

TABLE I

Effect of GaMδ on spleen cell surface markers[a]

Mice	Day	Antibody Injected	Percent (Median Fluorescence Intensity) of Spleen Cells with Surface				
			IgD	IgM	IgG	Ia	Thy-1.2
BALB/c	1	NI G IgG	55.3 (100)	55.8	3.8	55.1 (71)	
		GaMδ	<2.0	47.1	5.9	47.5 (125)	
	3	NI G IgG	50.0 (75)	52.5	3.6	49.0 (83)	33.6
		GaMδ	6.3 (32)	64.3	2.8	63.5 (156)	21.8
	5	NI G IgG	52.3 (113)	55.7	3.9	49.8 (86)	33.1
		GaMδ	2.3 (30)	55.9	6.2	52.8 (207)	31.2
	6	NI G IgG	37.5 (82)	37.9	2.0	40.7 (63)[b]	36.4
		GaMδ	3.7 (45)	46.1	12.3	65.9 (236)[b]	24.7
	7	NI G IgG	47.8 (99)	47.9	4.6	49.2 (80)[c]	37.3
		GaMδ	21.6 (14)	42.9	23.8	71.2 (150)[c]	20.5
	10	NI G IgG	47.4 (62)	52.7	5.0	60.2 (78)[b]	26.4
		GaMδ	30.9 (42)	45.6	29.2	72.4 (87)[b]	20.8
	13	NI G IgG	54.4 (72)	56.7	4.1		31.5
		GaMδ	37.5 (62)	40.0	13.9		38.1
	14	NI G IgG	44.1 (128)	43.4	6.5	51.4 (107)	40.5
		GaMδ	31.3 (135)	31.6	7.9	42.1 (113)	48.5
BALB/C nu/nu	2	NI G IgG	68.0 (87)	71.8	8.6	79.9	
		GaMδ	25.4 (14)	66.8	6.1	63.5	
	7	NI G IgG	79.5 (81)	78.9	6.6	52.5 (116)	
		GaMδ	18.1 (53)	52.2	11.2	44.8 (221)	
BALB/c tolerized to NI G IgG	2	NI G IgG	59.0 (80)	59.9		43.4 (69)	28.6
		GaMδ	6.4 (33)	56.2		52.5 (145)	30.5
	7	NI G IgG	47.2 (109)	41.1	5.6		41.3
		GaMδ	7.6 (10)	25.1	5.7		54.8
BALB/c	2	CBPC-101	42.0	47.7		38.7 (123)	36.2
		4.22aMδ[a]	<1.0	37.1		31.6 (249)	46.0
BCF₁	2	CBPC-101	44.4	53.4		45.5 (121)	32.0
		4.22aMδ[a]	24.5	50.5		41.9 (193)	33.3
BALB/c	7	CBPC-101	53.0 (123)	55.4	5.2		38.2
		4.22aMδ[a]	3.7 (22)	34.1	12.9		60.0
BCF₁	7	CBPC-101	55.2 (115)	56.3	3.6		48.9
		4.22aMδ[a]	36.4 (95)	49.3	4.0		48.1

[a] Suspensions of spleen cells pooled from at least three mice injected 1 to 14 days earlier with 800 μg of anti-δ or control antibody were stained with FITC-labeled antibodies specific for IgD, IgM, IgG, or Thy-1.2, or with A.TH anti-A.TL followed by FITC-RaMγ. These suspensions were analyzed for the percent of specifically stained cells and for the median fluorescence intensity (average brightness) of specifically stained cells (shown in parentheses) with an FACS II fluorescence-activated cell sorter.

[b] Stained with FITC-labeled hybridoma MKD6.

[c] Stained with FITC-labeled hybridoma 25-9.17.

The sIgG found on increased numbers of spleen cells 6 to 7 days after GaMδ injection could represent intrinsic membrane IgG and/or cytophilic IgG. Indeed, the possibility that the sIgG was entirely cytophilic initially seemed very likely, because GaMδ injected mice are producing anti-goat Ig antibodies 7 days after injection, and the resulting mouse anti-goat Ig-GaMδ complexes might be expected to bind to spleen cell Fc receptors and sIgD. Staining of spleen cells from these mice with an FITC-labeled rabbit anti-goat Ig antibody (a gift of Dr. Ellen Vitetta), however, failed to reveal the presence of more than trace amounts of goat IgG on the surface of these cells. In addition, when spleen cells from mice injected 7 or 13 days earlier with NI G IgG or GaMδ were stained with FITC-labeled RaMδ, RaMμ, or a mixture of both antibodies, the percentages of cells that were bound by the mixture of antibodies were nearly the sums of the percentages that were stained by either antibody alone (Table II). Thus, sIgM⁺ and sIgG⁺ spleen cells were predominantly separate populations. The sIgG⁺ spleen cells were most likely B rather than T lymphocytes, because the increase in the percentage of sIgG⁺ cells was accompanied

TABLE II

Surface IgM⁻ and surface IgG⁻ spleen cells are predominantly separate populations[a]

Day	Antibody Injected	Percent Spleen Cells with Surface			
		IgM	IgG	IgM or IgG	Thy-1.2
7	NI G IgG	48.0	3.9	50.9	33.3
	GaMδ	42.5	21.7	59.3	24.2
13	NI G IgG	56.7	4.1	59.1	29.8
	GaMδ	40.0	13.9	63.2	38.1

[a] Suspensions of pooled spleen cells from at least three mice injected 7 or 13 days earlier with 800 μg of GaMδ or NI G IgG were stained with FITC-labeled antibodies specific for IgG, IgM, or Thy-1.2, or with a mixture of anti-IgG and anti-IgM antibodies, and were analyzed for the percent of specifically stained cells with an FACS.

by an increase in the percentage of sIa⁺ cells and a fall in the percentage of sThy-1.2⁺ cells (Tables I and II). If the presence of sIgG on B lymphocytes resulted from the binding of IgG-containing immune complexes to IgG Fc receptors, sIgM⁺ B cells, which bear such receptors, would be expected to also bear sIgG. The finding that almost all sIgM⁻ B cells were sIgG⁻

therefore suggested that the sIg on the sIgG⁻ B cell population might be intrinsic rather than cytophilic.

To investigate further whether the IgG appearing on lymphoid cells 7 days after GaMδ injection was intrinsic or cytophilic, mesenteric lymph node and spleen cells from these and control mice were surface-labeled with ¹²⁵I by using the lactoperoxidase technique, and aliquots of NP-40 extracts of these labeled cells were directly immunoprecipitated with Staph A, or Staph A plus RaMIg. Direct Staph A immunoprecipitates would be expected to contain only IgG, because Staph A binds murine IgG2 and IgG3 avidly, IgG1 variably, and IgM, IgD, and IgA poorly if at all (19, 28, 32, 33), whereas Staph A plus RaMIg immunoprecipitates would be expected to include all isotypes of murine sIg. In addition, aliquots of Staph A-absorbed NP-40 extracts of ¹²⁵I-surface-labeled mesenteric lymph node cells from mice that had been injected 7 days earlier with control antibody (GaF) or GaMδ were immunoprecipitated with Staph A plus RaMIg, RaMδ, RaMμ, or RaKLH (control). All immunoprecipitated sIg were eluted from Staph A, reduced, and analyzed by SDS-PAGE. This procedure allows intrinsic membrane IgG to be distinguished from cytophilic serum IgG, because the γ-chain of the former has an apparent m.w. approximately 10,000 daltons greater than the latter (34, 35). Minimal sIg from the control lymph node cells bound directly to Staph A (Fig. 1A, line 1) whereas lymph node cells of GaMδ-treated mice bore at least two electrophoretically separable sIg that bound to protein A-Sepharose (Fig. 1A, lane 6). One of these had a heavy chain with a mobility identical to that of secreted γ-chain; the other had a mobility slightly faster than that of δ-chain, and thus corresponded to the previously described cell surface γ-chain. Surface μ-chains were precipitable from extracts of lymph nodes cells from both control and GaMδ-treated mice (Fig. 1A, lines 4 and 9, respectively) whereas surface δ-chain was precipitable from the extract of lymph node cells from control but not GaMδ-treated mice (Fig. 1A, lines 3 and 8). It is notable that RaMIg plus Staph A precipitated a heavy

chain with the mobility of surface γ-chain from an NP-40 extract of mesenteric lymph node cells that had been absorbed with Staph A; this probably represents γ₁, because the binding of murine IgG1 to Staph A is often incomplete. As shown in Figure 1B, the SDS-PAGE electropherograms of reduced RaMIg plus Staph A immunoprecipitates of surface-labeled spleen cells from mice injected 7 days previously with control antibody (lane 1) or GaMδ (lane 2) resembled those prepared from mesenteric lymph node cells. These data indicate that at least a considerable amount of the IgG on spleen cells from GaMδ-treated mice appears by a m.w. criterion to be intrinsic. It is uncertain whether the sIgG represented by the γ-chain band with a mobility characteristic of serum γ-chain represents cytophilic IgG, IgG in the process of being secreted, or a second subset of membrane IgG. In this regard, it is of interest that tumor cells that bear membrane IgG but do not secrete IgG demonstrate only the larger γ-chain by SDS-PAGE analysis, whereas SDS-PAGE characterization of surface-labeled γ-chain from tumor cells that bear membrane IgG and secrete IgG demonstrates both the heavy and light γ-chains. This finding has recently been shown to be due to molecules that contain both the membrane and secreted forms of γ-chain that are present on the surface of these IgG-secreting cells (J. W. Goding, presented at The New York Academy of Sciences Conference on Immunoglobulin D: Structure and Function, January, 1982).

It was also possible that the light γ-chain band apparent on SDS-PAGE electropherograms of spleen cell sIg from GaMδ-treated mice represented the heavy chain of GaMδ that had bound to these cells, because goat and mouse serum γ-chains have identical electrophoretic mobilities. This was investigated by studying the SDS-PAGE electrophoretic characteristics of spleen cell sIg from mice injected with 800 μg of RaMδ instead of GaMδ. Although RaMδ and GaMδ have similar stimulatory effects when injected i.v. into mice, the γ-chain of rabbit IgG has a faster electrophoretic mobility than the γ-chain of mouse
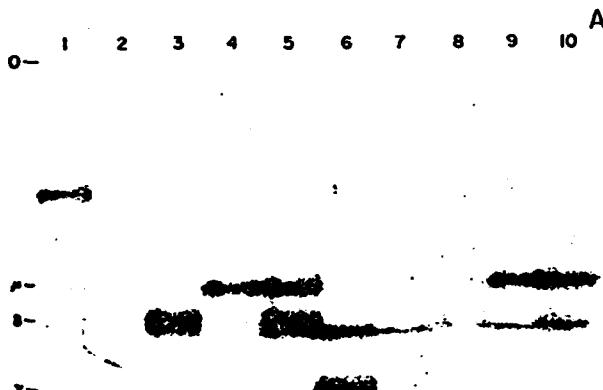
Figure 1. A, pooled mesenteric lymph node cells from three mice injected 7 days earlier with 800 μg of control antibody (GaF) or GaMδ were surface-labeled with ¹²⁵I and NP-40 extract. NP-40 extracts were absorbed with Staph A, after which aliquots were immunoprecipitated with Staph A plus RaKLH (control), RaMδ, RaMμ, or RaMIg. Labeled Ig that had absorbed directly to Staph A or to Staph A plus RaKLH, RaMδ, RaMμ, or RaMIg was eluted, reduced, and analyzed by slab discontinuous SDS-PAGE. The gel was stained to identify the position of a γ-chain marker, and then was developed by radioautography. Bands in lanes 1-5 and 6-10 represent labeled Ig heavy chains from mesenteric lymph node cells from mice injected with GaF or GaMδ, respectively, precipitated with Staph A alone (lanes 1 and 6) or, after Staph A absorption, with Staph A plus RaKLH (lanes 2 and 7), Staph A plus RaMδ (3 and 8), Staph A plus RaMμ (4 and 9), or Staph A plus RaMIg (5 and 10). Markers represent gel origin (O) and the electrophoretic positions of heavy chains of mouse cell surface IgM (μ) or IgD (δ) or secreted IgG (γ). Ig light chains have been run off the gel. B, pooled spleen cells from three mice injected 7 days earlier with 800 μg of GaF (lanes 1 and 2) or GaMδ (lanes 3 and 4) were surface-labeled, NP-40-extracted, immunoprecipitated with RaMIg plus Staph A (lanes 1 and 3) or RaKLH plus Staph A (lanes 2 and 4), eluted, reduced, and analyzed by SDS-PAGE. κ marker represents the electrophoretic position of mouse κ-chain.

TABLE III

Stimulation of immunoglobulin secretion by GaMδ

Mice	Day	Antibody Injected	Percent of Spleen Cells[a] with intracytoplasmic		CPM of ^{3}H-Leucine incorporated into Secreted[b]		
			IgM	IgG	μ	γ	Total Protein
BALB/c	5	NI G IgG	0.9 (1.45)[c]	<0.2			
		GaMδ	2.2 (1.53)	0.4 (2.03)			
	6	NI G IgG	0.3 (2.07)	0.5 (1.50)			
		GaMδ	4.9 (1.04)	3.1 (1.57)			
	7	NI G	0.4 (1.09)	0.5 (2.14)	643 (2.56)[c]	1,480 (1.17)	216,000 (1.54)
		GaMδ	2.5 (1.67)	15.7 (1.28)	3,680 (1.57)	70,300 (1.29)	869,000 (1.47)
	10	NI G IgG	0.9 (1.14)	1.3 (1.12)			
		GaMδ	0.5 (1.23)	4.9 (1.97)			
	13	NI G IgG	0.5 (1.23)	0.3 (2.19)	3,100 (1.81)	722 (2.54)	
		GaMδ	0.8 (1.34)	1.0 (2.30)	2,980 (1.98)	2,630 (1.71)	
BALB/c nu/nu	7	NI G IgG	2.6 (1.29)	0.8 (2.90)	7,100	2,000	83,800
		GaMδ	1.7 (2.56)	1.2 (1.45)	23,000	4,060	174,000
BALB/c tolerized to NI G IgG	7	NI G IgG	0.5 (1.24)	0.6 (1.47)	860 (1.19)	955 (1.53)	161,000 (1.12)
		GaMδ	1.2 (1.21)	1.5 (1.42)	442 (1.28)	926 (1.53)	159,000 (1.50)
BALB/c	7	CBPC-101	1.1 (2.93)	0.3 (1.51)	7,610	2,220	
		4.22aMδ	5.0 (1.73)	6.4 (1.96)	48,800	65,400	
BCF₁	7	CBPC-101	0.9 (1.20)	0.3 (1.78)	12,140	2,000	
		4.22aMδ	0.7 (2.73)	0.4 (2.08)	3,720	2,200	

[a] Cytocentrifuge preparations of spleen cells from mice injected 5 to 13 days earlier with anti-δ or control antibody were fixed, stained with FITC-RaMμ or FITC-RaMγ, and were examined with a Leitz Ortholux phase/fluorescence microscope for percentage of cells with bright intracytoplasmic fluorescence. At least 500 cells were examined on each slide.

[b] Spleen cells from mice injected 5 to 13 days earlier with anti-δ or control antibody were incubated overnight in leucine-free RPMI 1640, supplemented with ^{3}H-leucine (0.1 mCi/ml; 4 × 10⁶ spleen cells/ml) for 18 hr. Supernatants of cell cultures were dialyzed and aliquots were immunoprecipitated with RaMIg antibody plus Staph A. Washed immunoprecipitates were eluted, reduced, and analyzed by SDS-PAGE. SDS-PAGE gels were fractionated with a Gilson gel mincer; fractions were suspended in Aquasol and counted by scintillation spectroscopy to determine ^{3}H-cpm in μ- and γ-chain peaks. Aliquots of dialyzed supernatants were also precipitated with cold 10% trichloroacetic acid, filtered onto cellulose discs, suspended in Aquasol, and counted by scintillation spectroscopy. All cpm are normalized to 1-ml volumes of dialyzed supernatant.

[c] Geometric mean (geometric standard deviation) of determinations made with spleen cells from three to five mice. Where no figures in parentheses are shown, results were determined with pooled spleen cells from three to five mice.

serum IgG and thus can be differentiated by SDS-PAGE. SDS-PAGE electropherograms of reduced ^{125}I-labeled spleen cell sIg from mice injected 7 days previously with RaMδ demonstrated clear bands characteristic of heavy and light mouse γ-chain, but no bands characteristic of rabbit γ-chain (data not shown); thus little or no RamΔ bound to the surface of such spleen cells.

Effects of GaMδ on Ig secretion. The observation that GaMδ induced the differentiation of sIgM⁺ lymphocytes into sIgG⁺ cells suggested the possibility that the same antibody also stimulated differentiation of resting B cells into Ig-secreting cells. This was investigated by staining spleen cells for cIgM and cIgG, by measuring the incorporation of ^{3}H-leucine into IgM, IgG, and protein by cultured spleen cells, and by measuring serum Ig levels of mice injected with GaMδ or control goat Ig. As shown in Table IV, a slight increase in the percentage of spleen cells with cIgM was noted 5 days after GaMδ injection, but no increase in the percentage of cIgG⁺ cells was seen at this time. By 6 days after GaMδ injection, however, considerable increases in the percentages of both cIgM⁺ and cIgG⁺ cells were noted, and by day 7, the percentage of cIgG⁺ cells had increased to over 30 times the control value. At this time, greater than 20% of the splenic B cell population was cIgG⁺. The majority of cIgG⁺ cells appeared microscopically to be blasts (large cells with a central nucleus and a high nuclear to cytoplasmic ratio) rather than mature plasma cells. The increase in the percentage of cIg⁺ cells was T-dependent and carrier-dependent. Neither congenitally athymic mice nor mice tolerized to goat IgG generated increased percentages of cIg⁺ spleen cells in response to GaMδ injection (Table III). In addition, while BALB/c mice generated considerable percentages

TABLE IV

Effect of GaMδ on serum immunoglobulin levels[a]

Mice	Antibody Injected	Serum Ig Concentration (mg/dl)[b]		
		IgM	IgG1	IgG2a
BALB/c	NI G IgG	38.1 (1.02)	174 (1.54)	178 (1.63)
	GaMδ	68.9 (1.41)	1,220 (1.12)	344 (1.55)
BALB/c tolerized	NI G IgG	38.3 (1.33)	337 (1.64)	223 (1.38)
	GaMδ	23.5 (1.27)	216 (2.04)	195 (1.02)

[a] Sera were obtained from normal BALB/c mice as well also BALB/c mice tolerized to goat IgG 7 days after injection of 800 μg of NI G IgG or GaMδ, and were analyzed for IgM, IgG1, and IgG2a concentration by the radial immunodiffusion technique.

[b] Geometric mean (geometric standard deviation) of sera from three mice.

of cIgM⁺ and cIgG⁺ spleen cells in response to 800 μg of 4.22aMδ, BCF₁ mice showed no such increase (Table III).

Because it was possible that the intracytoplasmic staining technique was detecting cells that were synthesizing but not secreting Ig, the incorporation of ^{3}H-leucine into IgM and IgG in cell culture supernatants was studied as a direct means of quantitating secreted Ig and comparing it to total protein secretion. As shown in Table III, spleen cells cultured 7 days after GaMδ injection incorporated six times more ^{3}H-leucine into secreted μ-chain, 48 times more ^{3}H-leucine into secreted γ-chain, and four times more ^{3}H-leucine into total secreted protein than did an equal number of spleen cells from control mice. Thus, the GaMδ-stimulated increase in IgG secretion was greatly in excess of the increase in total protein secretion. Considerable increases in Ig secretion were also noted with spleen cells from BALB/c mice injected with 4.22aMδ; however, neither spleen cells from goat IgG-tolerant mice injected with GaMδ nor BCF₁ mice injected with 4.22aMδ demonstrated increased Ig secretion (Table III). Although spleen cells from

congenitally athymic mice injected 7 days previously with GaMδ did show increased incorporation of ^{3}H-leucine into secreted Ig, the increase was proportionate to an increase in total protein secretion, and probably represents something other than the appearance of increased numbers of antibody-secreting cells.

Measurement of serum Ig levels from GaMδ-injected and control mice gave results consistent with the intracytoplasmic staining and internal labeling studies. As shown in Table IV, sera drawn from BALB/c mice 7 days after GaMδ injection had IgG1 levels that were sevenfold increased over control values, as well as slightly increased IgG2a and IgM levels. Serum IgG2b and IgA levels were not increased by GaMδ injection (data not shown). As expected, mice tolerized to goat IgG before GaMδ injection did not show increased serum IgG or IgM levels.

The GaMδ-induced increase in cIg secretion is polyclonal. The above data show that the in vivo injection of GaMδ induces a marked increase in IgG secretion that appears to involve the differentiation of a substantial percentage of splenic B lymphocytes into antibody-secreting cells. Although the large percentage of cIg$^+$ cells found strongly suggested that this was a polyclonal process, we felt it necessary to investigate the possibility that the increase in Ig secretion was an antigen-specific response directed entirely to the immunogen (goat Ig) because previous anti-δ stimulation studies with monkeys, rats, and mice had demonstrated this finding (18, 36–41). As a first step, cytocentrifuge preparations of spleen cells from mice injected 7 days earlier with GaMδ were stained with FITC-labeled NI G IgG and were examined for the presence of cells with intracytoplasmic fluorescence. While 10 to 20% of the cells in these preparations demonstrated intracytoplasmic fluorescence after staining with FITC-RaMγ and FITC-RaMμ, less than 0.2% of cells in any preparation showed intracytoplasmic fluorescence after staining with FITC-NI G IgG. This result was not definitive, however, because it is uncertain whether cells synthesizing anti-goat Ig antibodies of low affinity would be detected by this technique. For this reason, the ability of goat Ig to absorb Ig from the sera of GaMδ-inoculated mice was also examined. Sera from each of two mice injected 8 days previously with 800 μg of NI G IgG or GaMδ were incubated overnight with equal volumes of either whole goat serum, heat-insolubilized goat IgG, or saline, centrifuged to remove insoluble material, and then were analyzed by radial immunodiffusion for IgG1 content. None of these absorptions decreased serum IgG1 levels by more than 5% (data not shown). This result strongly suggested that GaMδ induced polyclonal IgG1 secretion. It did not, however, eliminate the possibility that GaMδ also stimulated considerable specific anti-goat Ig antibody se-

cretion within 7 days of injection, because secreted anti-goat Ig might have been absorbed in vivo by injected GaMδ. To investigate this possibility 2-ml aliquots of supernatants of spleen cells cultured in ^{3}H-leucine-containing medium were allowed to adsorb for 30 min to a 1 × 9 cm column of goat serum globulin-Sepharose, which had approximately 10 mg of a 50% saturated $(NH_4)_2SO_4$ cut of goat serum bound per milliliter of Sepharose. This column was then washed with two column volumes of 0.1 M Tris, pH 8.3, (absorbed fraction) and was eluted with 3.5 M $MgCl_2$ (eluted fraction). Ovalbumin was added to the eluted fraction to a concentration of 1 mg/ml, after which it was dialyzed against 0.1 M Tris, pH 8.3. Both absorbed and eluted fractions were then concentrated to 2-ml volumes with an Amicon Minicon concentrator. RaMlg plus Staph A immunoprecipitates were prepared from bound and eluted fractions and were analyzed for cpm of μ- and γ-chains as described earlier. As shown in Table V, approximately one-third of the total cpm of μ- and γ-chains were in the eluted fraction. These results do not necessarily mean that one-third of the Ig secreted by spleen cells from GaMδ-stimulated mice was goat serum globulin-specific; although the high antigen to antibody ratio, long absorption period, and limited wash of the column allowed binding of low affinity antibody to the goat serum globulin column, it also maximized nonspecific binding. To estimate the extent to which nonspecific binding did occur, a similar experiment was performed with the ^{3}H-labeled supernatant of cultured spleen cells from BALB/c mice injected 7 days before sacrifice with 4.22aMδ^a. Because this antibody is a murine Ig, it is unlikely that cells stimulated by it would secrete an appreciable amount of antibody specific for goat serum globulins; however, approximately 15% of the IgM and 10% of the IgG in this supernatant bound to goat serum globulin-Sepharose under the conditions used (Table V). Thus, it may be estimated that as much as 20% (33% minus 13%) of the Ig secreted by GaMδ-stimulated mice represents a specific response to goat serum globulins, while approximately 80% represents a true polyclonal response.

To investigate further whether the GaMδ-stimulated antibody response was in large measure polyclonal, the in vivo effects of GaMδ on the generation of cells secreting antibody to an antigen unrelated to goat serum globulins was studied by comparing the number of direct anti-TNP plaque-forming cells (PFC) in a modified Jerne plaque assay (42, 43) with the total number of IgM-secreting cells in a protein A reverse plaque assay (44). As shown in Table VI, 7 days after its injection, GaMδ enhanced the numbers of both kinds of PFC by similar factors (seven to ninefold), whereas 14 days after GaMδ injection neither the frequency of IgM-secreting cells nor that of IgM anti-TNP-secreting cells was increased. Similar results were seen in three additional experiments. This demonstration that GaMδ stimulates the differentiation of cells secreting antibody to an unrelated antigen provides further evidence for the polyclonal nature of the GaMδ-induced antibody response.

TABLE V

Adsorption of internally labeled immunoglobulin secreted by spleen cells from GaMδ and 4.22aMδ-stimulated mice with goat serum-Sepharose[a]

Antibody Injected	^{3}H-Leucine cpm			
	Adsorbed to Goat Serum-Sepharose		Not Adsorbed to Goat Serum-Sepharose	
	μ	γ	μ	γ
GaMδ	14,000	129,000	30,000	255,000
4.22aMδ^a	3,500	5,090	23,000	51,100

[a] Pooled, dialyzed, ^{3}H-leucine internally labeled culture supernatants from groups of three mice that had been injected 7 days earlier with 800 μg of GaMδ or 4.22aMδ^a were prepared as described in Table IV. Labeled supernatants were allowed to adsorb to a column of the 50% saturated $(NH_4)_2SO_4$ cut of goat serum bound to CNBr-activated Sepharose, after which columns were briefly washed and then eluted with 3.5 M $MgCl_2$. The eluted adsorbed fraction was dialyzed against 0.1 M Tris, pH 8.3, after which both it and the fraction that did not adsorb to the goat serum-Sepharose were concentrated in Amicon Minicon concentrators and were immunoprecipitated with RaMlg plus Staph A. CPM of μ- and γ-chains in both fractions were determined as described in Table IV.

TABLE VI

GaMδ induces similar increases in the frequencies of total IgM-secreting cells and IgM anti-TNP-secreting cells[a]

7	NI G IgG	365 ± 25^b	7 ± 1
	GaMδ	2,546 ± 911	67 ± 16
14	NI G IgG	545 ± 145	<5
	GaMδ	605 ± 125	<5

[a] Spleen cells from BALB/c mice injected 7 days earlier with 800 μg of NI G IgG or GaMδ were plaqued against protein A-coated sheep erythrocytes in the presence of RaMμ antibody or against TNP-haptenated sheep erythrocytes to determine the total numbers of IgM-secreting cells or IgM anti-TNP-secreting cells per 10^4 spleen cells plaqued.

[b] Arithmetic mean ± standard deviation of PFC from three mice.

TABLE VII

IgG subclass distribution of spleen cells with surface or intracytoplasmic IgG 7 days after anti-δ injection[*]

Expt.	Antibody Injected	Percent Spleen Cells with Surface				Percent Spleen Cells with Intracytoplasmic			
		IgG	IgG1	IgG2	IgG3	IgG	IgG1	IgG2	IgG3
1	NI G IgG	4.1	1.2	1.5	1.5	0.5 (2.14)[*]			
	GaMδ	27.2	18.3	7.4	2.8	15.7 (1.28)	15.0 (1.15)	0.5 (1.42)	<0.2
2	NI G IgG	3.9	1.1	1.0	1.0				
	GaMδ	21.7	12.3	1.0	1.0				
3	NI G IgG		2.3	2.8			0.9 (2.05)	0.4 (3.65)	
	GaMδ		18.3	3.9			9.5 (1.11)	1.4 (1.62)	
4	RaKLH	4.6	2.6	1.2	2.1				
	RaMδ	26.1	25.0	3.4	2.3				
5	CBPC-101	5.2	1.7	1.5	<1.0	0.3 (1.51)			
	4.22aMδ[a]	12.9	6.1	5.3	<1.0	6.4 (1.96)	5.1 (1.96)	0.5 (2.27)	

[*] Spleen cells from mice injected 7 days before sacrifice with 800 μg of anti-δ or control antibody were analyzed as described in Tables II and IV for surface or intracytoplasmic IgG1, IgG2, or IgG3, by using affinity-purified F(ab')₂ fragments of rabbit antibodies specific for these isotypes. Surface Ig staining was performed with spleen cells from individual mice.

[a] Geometric mean (geometric standard deviation) of values obtained from three mice.

The polyclonal antibody response induced by GaMδ is primarily of the IgG1 isotype. Experiments were performed to determine the subclass distribution of sIgG⁺ and cIgG⁺ spleen cells from GaMδ-treated mice, as well as the possible relationship between sIgG⁺ and cIgG⁺ cells. As shown in Table VII, a large majority of the sIgG⁺ cells, and almost all cIgG⁺ cells were of the IgG1 isotype. This was true regardless of whether GaMδ, RaMδ, or 4.22aMδ[a] was the anti-δ antibody used. This corresponded to the assay of serum Ig levels, mentioned above, which indicated that GaMδ induced a greater increase in IgG1 levels than in IgG2a or IgG2b levels.

To investigate whether there was a relationship between cells that bore sIgG and cells that contained cIgG, spleen cells from mice injected 7 days previously with GaMδ were stained with FITC-RaMδ and were sorted with an FACS into sIgG⁺ and sIgG⁻ populations. Reanalysis of the sorted populations indicated that only 6% of the "sIgG⁻" population bore sIgG, whereas sIgG could be detected on 72% of the cells in the "sIgG⁺" population. Cells from both sorted populations were cytocentrifuged onto glass slides, fixed, and stained for cIg with FITC-RaMγ and FITC-RaMμ. The percentages of cIgG⁺ cells in the sIgG⁺ and sIgG⁻ populations were 16.2 and 0.9%, respectively; the percentages of cIgM⁺ cells in the sIgG⁺ and sIgG⁻ populations were, respectively, 3.2 and 10.8%. Thus, the majority of cIgG⁺ cells 7 days after GaMδ injection also bear sIgG, whereas the cIgM⁺ cells are mostly sIgG⁻. This association between sIg and cIg of one isotype and the suggested mutual exclusivity of the cIgG⁺ and cIgM⁺ populations corresponds to the apparent lack of overlap between the sIgM⁺ and sIgG⁺ populations mentioned above.

Specificity of GaMδ-induced polyclonal activation. Because GaMδ can form immune complexes with serum and cell surface IgD, and 400 μg of heat-aggregated rabbit IgG has been reported to induce a polyclonal antibody response when injected *in vivo* (45), we immunized mice i.v. with 400 μg of heat-aggregated goat IgG and examined their spleen cells 5 and 7 days later for the presence of sIgG⁺ cells and incorporation of ³H-leucine into IgM and IgG. Little or no increase was found, although GaMδ stimulated a strong polyclonal response (data not shown). The ability of a monoclonal rat antibody with specificity for the ThB determinant (present on almost all B lymphocytes and approximately 50% of thymocytes but not on mature T cells) (46) to stimulate polyclonal B lymphocyte activation was also investigated. Intravenous injection of 800 μg of this antibody substantially diminished the amount of the ThB determinant on spleen cells both 2 and 7 days after

injection; however, as previously reported, injection of this antibody failed to raise B cell sIa levels. In contrast to three antibodies with δ-chain specificity (4.22aMδ[a], RaMδ, and GaMδ) this antibody failed to induce an increase in the percent of spleen cells with sIgG or cIg (data not shown).

Collapse of the activated immune state. Spleen cells from mice injected more than 7 days previously with GaMδ were evaluated for the presence of sIg and cIg. The percentage of cIgG⁺ cells was considerably decreased by 10 days after GaMδ administration and had almost returned to baseline at 14 days after GaMδ administration (Table II). Investigation of bone marrow cells 14 days after GaMδ injection also failed to show an increased amount of Ig secretion, determined by internal labeling and SDS-PAGE studies (data not shown). The increase in the percentage of sIgG⁺ cells in spleen outlasted the increase in cIg⁺ cells, although it too was greatly decreased 14 days after GaMδ injection (Table I). In addition, sIgG⁺ spleen cells detected 13 days after GaMδ injection were predominantly small lymphocytes rather than the sIgG⁺ blasts detected 7 days after GaMδ injection. The percentages of B cells (sIa⁺ cells) 13 and 14 days after GaMδ injection were decreased below the baseline value; this was particularly apparent for the sIgM⁺ sIgD⁺ B cell subset (Table I). An increase in the percent of Thy-1.2⁺ spleen cells 14 days after GaMδ injection corresponded to the decrease in the percent of splenic B cells.

DISCUSSION

The data presented here confirms our previous report that *in vivo* injection of anti-δ antibody induces an increase in B cell sIa (31), and provides the initial demonstration that the same treatment induces a polyclonal IgM to IgG switch in B cell surface isotype as well as the simultaneous appearance of large numbers of Ig-secreting cells. Although the increase in B cell sIa appears to be a direct effect of the sIgD-anti-δ interaction, the sIg isotype switch and the differentiation of B lymphocytes into antibody-secreting cells both involve T lymphocytes and require recognition of the anti-δ molecule as foreign. The observation that GaMδ predominantly stimulates IgG1 secretion is consistent with a T-dependent model of anti-δ activation of antibody secretion, because soluble, T-dependent antigens stimulate a predominantly IgG1 response, whereas T-independent antigens stimulate significant IgG2 and IgG3 antibody responses (47, 48).

The data presented in this and the accompanying paper suggest that antigen-induced B cell activation proceeds in at least two stages. In the first stage, increases in B cell sIa, B

cell size, and the rate of B cell DNA synthesis can be seen. As described in the companion paper, these changes lead to activation of T cells specific for determinants on the anti-δ molecule. The second stage results in further stimulation of the activated B lymphocyte by the activated T lymphocyte or its products. The increase in B cell sIa may facilitate both antigen presentation to T lymphocytes and B lymphocyte acceptance of T cell help. At least two models of B cell activation that are consistent with our data offer mechanisms for the specificity of antibody responses to T-dependent antigens. In one, carrier-specific T helper factors would bind to antigen (or anti-δ) molecules that had bound to sIg and stimulate differentiation towards antibody secretion (49). In a second model hapten specificity would be restricted to the initial stages of B cell activation (i.e., helper factors would be generated that are not carrier-specific, but only those B lymphocytes initially activated by antigen or anti-Ig cross-linking of sIg could respond to these factors by generating clones of antibody-secreting cells) (50). Because experimental evidence supports the existence of both carrier-specific and nonspecific helper factors, there is no reason not to believe that both mechanisms of T-dependent B cell activation play a role in the phenomena we have described.

The extent of B cell activation induced by GaMδ injection strongly suggested that the immune response was polyclonal. Our data indicate that although most control mice have less than 10^5 antibody-secreting cells out of approximately 10^8 total spleen cells, mice injected 7 days earlier with GaMδ often have 5×10^7 antibody-secreting cells in a spleen containing 3 to 4 $\times 10^8$ nucleated cells. The polyclonal nature of the GaMδ-induced antibody response was confirmed by studies that showed most of the Ig produced by stimulated mice lacked specificity for goat serum globulins, and that the increases in the frequencies of total IgM-secreting spleen cells and IgM anti-TNP-secreting spleen cells were nearly identical. The determination that the GaMδ-stimulated Ig response was polyclonal rather than specific for goat Ig indicates that a ligand-sIgD interaction, in the absence of a ligand-sIgM interaction, can both induce B cells to proliferate and initiate a series of events that leads B lymphocytes to differentiate into antibody-secreting cells. This result suggests that the correct interpretation of previous experiments in which anti-μ antibodies blocked a wide range of in vitro antibody responses (13–18) is that the anti-μ-sIgM interaction blocked antibody production through a direct inhibitory effect on B cell differentiation rather than by the prevention of a required antigen-sIgM interaction.

Our results differ from previous in vitro studies that indicated anti-δ antibodies could induce B cells to differentiate into antibody-secreting cells only when they were further stimulated by T-dependent helper factors (10–12), treated with proteolytic enzymes, or thoroughly washed (51); these additional treatments proved unnecessary for in vivo activation of antibody production. These differences may result from anti-δ having less of a direct inhibitory effect on B cell differentiation in vivo than in vitro, and/or a greater ability of anti-δ to stimulate activation of helper T cells under in vivo conditions. In addition, there may be qualitative differences between the in vitro and in vivo mechanisms of anti-δ-stimulated B cell activation; anti-Ig plus mitogen-stimulated helper factor fail to induce spleen cells from mice with the CBA/N immune defect to proliferate or differentiate into antibody-secreting cells in vitro (10), whereas GaMδ injected into these mice induces both (L. Muul et al., manuscript in preparation).

Our data do not establish that a ligand-sIgD interaction has unique B cell-activating effects or is required for B lymphocyte

activation. In vitro studies have established that anti-μ antibodies, like anti-δ antibodies, can stimulate B lymphocytes to increase their sIa (31), to proliferate (2–6, 8, 11), and when further stimulated, to differentiate into antibody-secreting cells (10). Furthermore, antigens injected into mice that lack sIgD⁺ B lymphocytes as a consequence of injection from birth with rabbit anti-δ antibodies still make good antibody responses to sheep erythrocytes (SRBC), TNP-SRBC, TNP-KLH, and TNP-Ficoll (52, 53), although they fail to respond to injected GaMδ (E. S. Metcalf et al., manuscript in preparation). Thus, it seems probable that ligand-sIgM and ligand-sIgD interactions can have similar B cell-activating effects. We have previously summarized evidence that leads us to believe that an important functional difference between sIgM-ligand and sIgD-ligand interactions is that sIgM-ligand interactions have a greater direct inhibitory effect on the differentiation of B cells into antibody-secreting cells than do sIgD-ligand interactions (18).

The stimulatory effects of anti-Ig antibodies on B cell activation do not result simply from the binding of ligand to any cell surface molecule. The injection of 400 μg of heat-aggregated goat IgG or 800 μg of a monoclonal rat antibody to the ThB determinant failed to increase B cell sIa or induce B cells to proliferate or differentiate into antibody-secreting cells. The lack of effect of the latter antibody was not a consequence of its monoclonality because 4.22aMδ², a monoclonal antibody with relatively low affinity for IgD, was capable of inducing polyclonal B lymphocyte activation.

The rapid collapse of the activated immune system after the catabolism of injected GaMδ suggests, as discussed in our previous paper, that polyclonal activation may stimulate polyclonal suppression. Indeed, spleen cells taken from mice 7 days after GaMδ injection suppress a number of in vitro proliferative responses of spleen cells from normal mice (J. Mond et al., unpublished data). While the putative suppressor mechanisms decrease numbers of both sIgG⁺ and cIgG⁺ spleen cells substantially after day 7, the decrease in the number of cIgG⁺ cells occurs more rapidly. Because the persistent sIgG⁺ cells are small cIgG⁻ lymphocytes rather than the cIgG⁺ blasts seen 7 days after GaMδ injection, the former sIgG⁺ cell population may consist of memory B cells that are somewhat less susceptible to suppression than are antibody-secreting cells. Thus, the injection of mice with large doses of GaMδ antibody provides a polyclonal model of at least one mechanism of B cell activation by a T-dependent antigen that generates B cells that have undergone surface isotype switching and differentiation into antibody-secreting cells and possibly memory cells as well as activated T helper and possibly T suppressor cells in numbers that have previously been difficult to obtain. Our model, therefore, is useful both in elucidating B cell activation and in providing activated cell populations for further study. Our model, however, most likely illustrates only one of several T-dependent mechanisms that can lead to B lymphocyte activation. It is unlikely, for example, that the injection of very small quantities of antigen in adjuvant could directly induce an increase in sIa or an increase in the proliferation rate of an antigen-specific B cell population, yet such stimulation can generate strong antibody and memory responses. The factor-generating and antigen-presenting roles of macrophages may be greatly enhanced by the presence of adjuvant in such an immune response, and the triggering of B lymphocyte proliferation and differentiation may be fundamentally different from that studied in our system (i.e., when antigen is injected with adjuvant an antigen → macrophage → T cell → B cell stimulatory pathway may be followed, rather than the antigen → B

cell → T cell → B cell stimulatory pathway that we have postulated from our results). Studies in which relatively small doses of anti-δ antibodies are injected in adjuvant are currently being performed to investigate whether the B cell activation mechanisms involved can be manipulated to generate a polyclonal response.

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